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Plasticity Genes Do Not Modify Associations Between Physical Activity and Depressive Symptoms

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Objective: Physical activity is inversely associated with depression in adolescents, but the overall associations are fairly weak, suggesting individual differences in the strength of the associations. The aim of this study was to investigate whether plasticity genes modify the reciprocal prospective associations between physical activity and depressive symptoms found previously. ***Methods:*** In a prospective population-based study (N = 1,196), physical activity and depressive symptoms were assessed three times, around the ages of 11, 13.5, and 16. Structural Equation Modeling was used to examine reciprocal effects of physical activity and depressive symptoms over time. The plasticity genes examined were *5-HTTLPR, DRD2, DRD4, MAOA, TPH1, 5-HTR2A, COMT,* and *BDNF* . A cumulative gene plasticity index consisting of three groups (low, intermediate, and high) according to the number of plasticity alleles carried by the adolescents was created. Using a multigroup approach, we examined whether the associations between physical activity and depressive symptoms differed between the three cumulative plasticity groups, as well as between the individual polymorphisms. ***Results:*** We found significant cross-sectional and cross-lagged paths from physical activity to depressive symptoms and vice versa. Neither the cumulative plasticity index nor the individual polymorphisms modified the strengths of these associations. ***Conclusion:*** Associations between adolescents' physical activity and depressive symptoms are not modified by plasticity genes.

Keywords: physical activity, depressive symptoms, plasticity genes, genes, adolescents

Individuals carrying specific genotypes have a higher sensitivity to environmental influences than others (Belsky et al., 2009). Although research initially focused on the negative consequences of these genotypes in the context of the classic diathesis-stress framework, more recent work stresses the possibility that individuals who suffer most from negative experiences might also benefit most from environmental support or the absence of adversity (Belsky & Beaver, 2011; Ellis, Boyce, Belsky, Bakermans-Kranenburg, & van Ijzendoorn, 2011). This "for better and for worse" manner in which individuals are more responsive to envi-

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ronmental influences suggests that individuals who carry specific genotypes might benefit more from positive environmental influences (such as family or social support) in early life, while they might also be more vulnerable to negative environmental influences (such as child maltreatment and negative life events) in developing psychopathology later on. Genes thought to be responsible for this differential susceptibility have been labeled plasticity genes.

Belsky and Pluess (2009) proposed several genes to operate as plasticity genes including the serotonin transporter gene (*SCL6A4*), the dopamine receptor 2 and 4 genes (*DRD2 & DRD4*), the dopamine transporter gene (*DAT1*), the serotonin 2a receptor gene (*5HT2RA*), the catechol-O-methyl transferase gene (*COMT*), the monoamine oxidase gene (*MAOA*), and the tryptophan hydroxylase 1 gene (*TPH1*). Although these genes are not the only likely candidates, they have been shown most consistently to act as plasticity genes in the literature (for a review, see Belsky & Pluess, 2009). Belsky and Pluess proposed a cumulative genetic plasticity model, in which the susceptibility to particular environmental influences is hypothesized to increase with increasing numbers of plasticity alleles (Belsky et al., 2009; Belsky & Beaver, 2011).

In adults, inverse associations between physical activity (PA) and depressive symptoms have been shown previously (Penedo & Dahn, 2005), supporting the idea that engaging in PA can at least prevent the development of depressive symptoms. In adolescents, fewer studies have investigated this relationship, and the results have been inconsistent so far (Johnson & Taliaferro, 2011). A recent prospective study in older adults demonstrated reciprocal inverse relationships between PA and depressive symptoms (Lindwall, Larsman, & Hagger, 2011). They found that there was a stronger relationship between PA predicting later depressive symptoms than between depressive symptoms predicting later engagement in PA. We also have reported reciprocal prospective associations between PA and depressive symptoms in a cohort of Dutch adolescents (Stavrakakis, de Jonge, Ormel, & Oldehinkel, 2011). Associations were measured using a cross-lagged path analysis model involving three measurements. The associations found were significant but rather weak, indicating a high amount of unexplained variance. Part of this unexplained variance may be because of individual differences in both the potential benefits of PA in alleviating depressive symptoms and the negative effects of depressive symptoms on PA levels. A better understanding of these individual differences is needed to design effective interventions for treating affective disorders.

We hypothesized that the plasticity genes might underlie part of the assumed individual differences in the reciprocal associations between PA and depressive symptoms. In their review, Belsky and Pluess (2009), proposed a theoretical framework in which they suggest that these individuals might have lower thresholds for experiencing pleasure and/or displeasure that might result in differential susceptibility to environmental influences. Although this is speculative, two major neurobiological mediating mechanisms have been identified, namely the serotonergic (experience of displeasure) and dopaminergic systems (reward sensitivity and sensation seeking). These two systems have been implicated in the etiology of depression (Lapin & Oxenkrug, 1969; van Praag & Korf, 1970) but also have been shown to be influenced by PA (Dishman et al., 2006). Alterations in serotonin and noradrenaline levels have been suggested to be the main reason for the beneficial effects of PA on stress-resilience and mood (Dishman et al., 2006). Evidence for an effect of PA on the dopaminergic system comes mainly from animal research where treadmill running increased the release (Meeusen, Piacentini, & De Meirleir, 2001) and turnover of dopamine (Hattori, Naoi, & Nishino, 1994) in the striatum of rats. Increasing dopamine levels is known to be an effective way to improve mood (Lemke, Brecht, Koester, & Reichmann, 2006). Therefore, these plasticity genes might modify the reciprocal association between PA and depressive symptoms through their differential action on the serotonergic and dopaminergic systems.

To date, only three studies have tried to elucidate whether specific genotypes may be associated with the effects of PA on depressive symptoms in humans. Rethorst, Landers, Nagoshi, and Ross (2010) showed that after 30 min of exercise, carriers of a long allele of the *5-HTTLPR* exhibited greater reduction in depressive symptoms than individuals with two short alleles. In a cross-sectional study, Rethorst, Landers, Nagoshi, and Ross (2011) demonstrated that the *5-HTTLPR* gene modified the relationship between PA and depressive symptoms in a sample of 170 students. Finally, Mata, Thompson and Gotlib (2010) conducted a study in adolescent girls, and found that PA involvement reduced depressive symptoms in carriers of the Brain Derived Neurotrophic Factor (*BDNF*) met allele, but not in carriers of the val/val polymorphism. BDNF is a growth factor which is encoded by the *BDNF* gene and is abundant throughout the human body. Belsky et al. did not include the *BDNF* gene in their list of plasticity genes. However, because BDNF seems to be related to both PA (Goekint et al., 2010; Knaepen, Goekint, Heyman, & Meeusen, 2010) and depressive symptoms (Hashimoto, 2010; Verhagen et al., 2010), the *BDNF* gene was considered a plausible modifier of the reciprocal association between PA and depressive symptoms, and therefore was included in our study as well.

The aim of the present study was to investigate whether plasticity genes modified the reciprocal prospective associations between PA and depressive symptoms for several reasons. First, it is important to extensively test robustness of gene environment interactions (G × E), as this field seems to be plagued by nonconfirmations. For example, the G × E between the short *5-HTTLPR* allele and adversity on depression first reported by Caspi, Hariri, Holmes, Uher, and Moffitt (2003) has not been confirmed in two meta-analyses (Munafo, Durrant, Lewis, & Flint, 2009; Risch et al., 2009), but has been justified in a later qualitative review (Caspi et al., 2010) and in the most recent meta-analysis (Karg, Burmeister, Shedden, & Sen, 2011) . Thus, for the advancement in the field of G × E regarding PA and depressive symptoms, confirmation studies are of utmost importance. Second, previous investigations focused only on the unidirectional effects of PA on depressive symptoms (Mata et al., 2010; Rethorst et al., 2010; Rethorst et al., 2011). We extend this line of research by investigating possible G × E in the reciprocal association between PA and depressive symptoms using previously developed cross-lagged models in a multigroup analysis. Third, we extend the G × E literature regarding PA and depressive symptoms by using an index of cumulative plasticity (Belsky & Beaver, 2011; Belsky et al., 2009; Belsky & Pluess, 2009). Summarizing, in order to test whether the associations between PA and depressive symptoms were moderated by genetic polymorphisms, we performed multigroup analyses with regard to both cumulative plasticity index and individual polymorphisms on cross-lagged models of PA and depressive symptoms.

Methods and Materials

Design

Data were used from the TRacking Adolescents' Individual Lives Survey (TRAILS), a prospective cohort (three assessment waves) of Dutch adolescents. Permission for the study was obtained from the Dutch Central Committee on Research Involving Human Subjects. Further information on TRAILS objectives, main design, data collection, sample selection, and nonresponse can be found elsewhere (de Winter et al., 2005; Huisman et al., 2008). Parents gave informed consent in the first wave, while for the second and third wave informed consent was obtained from both the parents and the participants themselves.

Procedure

During the first assessment wave, parents or guardians were visited by TRAILS interviewers in their homes and were administered an interview. In addition, they were also asked to complete a questionnaire. During the second and third wave, parents had to complete a questionnaire which was sent through the mail. The adolescents filled out self-report questionnaires at school. Further information on TRAILS procedures can be found elsewhere (de Winter et al., 2005; Huisman et al., 2008).

Participants

In the first assessment wave (T1) 2,230 adolescents from a possible 2,935 (51% girls, mean age = 11.08, *SD* = 0.59) agreed to take part in TRAILS. The response rates at the second wave and third wave were 96.4% (*N* = 2,149; 51% girls, mean age = 13.65, *SD* = 0.53) and 81.4% (*N* = 1,816; 53.2% girls, mean age = 16.27, *SD* = 0.73) respectively. For the present study only participants with complete genetic data were included (*N* = 1,196, 52.3% girls). Individuals that did not have both parents born in the Netherlands were also excluded from the final analysis, because these single-nucleotide polymorphisms (SNPs) can differ between ethnic groups. This focus sample was still representative of a normal population of adolescents and the problems observed therein.

Measures

Depressive symptoms. Adolescents filled out the Affective Problems scale of the Youth Self Report (Achenbach, 1991b) for each assessment wave at school. Parents completed the parent version of the YSR the Child Behavior Checklist (Achenbach, 1991a) at home. The mean scores of the YSR and CBCL scales were used in the analyses. The Affective Problem scale contains 13 items which correspond to Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (*DSM–IV*) depressive symptoms (van Lang, Ferdinand, Oldehinkel, Ormel, & Verhulst, 2005). These items include sadness, loss of pleasure, crying, self-harm, suicidal ideation, feelings of worthlessness, guilt, loss of energy, overtiredness, eating problems, and sleeping problems; and are scored on a 3-point scale (0 = *never or not at all true*, 1 = *sometimes true*, and 2 = *very often or very true*). One of the sleep items ("I sleep more than most kids") was excluded because exclusion of this item increased the internal consistency of the scale (Bosch et al., 2009). Scores on the remaining 12 items were averaged to construct a total Depressive symptoms scale with an internal consistency (Cronbach's alpha) of 0.74 at T1, of 0.81 at T2, and 0.86 at T3.

PA. At T1 adolescents were asked to rate how many times per week they performed physical exercise (e.g., swimming, playing football, horse-riding) on a 5-point scale (0 = *never*, 1 = *once per week*, 2 = *two to three times per week*, 3 = *four to five times per week*, and 4 = *five to six times per week*). At T2 and T3, separate questions were asked for PA during the summer and the winter: "How many days in an average week in the summer/winter do you take part in physical activities?" and the responses were scored on an 8-point scale (0 = *never* to 7 = *seven days per week*). The answers to these two questions were averaged, and mean scores were used in the analysis. T1 responses (ranging from 0–4) were recoded into an 8-point scale using the monotonic R7 transform recommended by Little to achieve a similar metric for the PA measures at all measurement waves (Little, in press).

Plasticity Genes

The list of plasticity genes as proposed by Belsky and Pluess (2009) include *DRD2, DRD4, 5HTTLPR, MAOA, TPH1, 5-HTR2A, COMT*, and the *DAT1*. In our study, *DAT1* was excluded because it was not genotyped, while the *BDNF* gene was included for reasons explained in the Introduction. Following the criteria set by Belsky and Pluess (2009), the A1 allele of *DRD2*, the long version of *DRD4* (from 7 repeats to 10 repeats), the short allele of 5-*HTTLPR*, the 2R/3R alleles of *MAOA*, the A allele of *TPH1*, the T allele of the *5-HTR2A*, the val (G) allele of *COMT*, and the met allele of *BDNF* were defined as plasticity alleles.

DNA Extraction

Blood samples (*n* = 1,190) or buccal swabs (Cythobrush) (*n* = 275) were collected for DNA extraction using a manual salting out procedure as described by Miller, Dykes, and Polesky (1988).

Genotyping of BDNF, DRD2, COMT, TPH1, and 5-HTR2A

The *BDNF* SNP (rs6265), *DRD2*/TaqIA (rs1800497), *COMT*/val158met (rs4680), *TPH1* (rs179913), and *5-HTR2A* (rs6313) were genotyped on the Golden Gate Illumina BeadStation 500 platform (Illumina, San Diego, CA) according to the manufacturer's protocol. Call rates were: 81% for *BDNF*, 100% for *DRD2*, 95% for *COMT*, 100% for *TPH1*, and 100% for *5-HTR2A*. All DNA samples could be amplified, and concordance between DNA replicates (*n* = 53) showed a 100% genotyping accuracy (Nederhof et al., 2010). Data cleaning was completed according to recommended procedures (Steptoe, 2010). All SNPs were well within Hardy-Weinberg equilibrium, with HWE *p* values ranging between .42 and .52.

Genotyping Length Polymorphisms of 5-HTTLPR, DRD4, and MAOA

Genotyping of the length polymorphisms (LP) *DRD4, MAOA, HTTLPR*, and SNP rs25331 (A/G SNP in *L HTTLPR*) was done at

the Research lab for Multifactorial Diseases within the Human Genetics department of the Radboud University Nijmegen Medical Centre in Nijmegen, The Netherlands. Genotyping of the *HTTLPR* polymorphism in the promoter region of *SLC6A4* (*5-HTT, SERT*) gene was performed by simple sequence length analysis. Call rate was 91.6%. A custom-made TaqMan assay (Applied Biosystems) was utilized to genotype the single nucleotide substitution (A to G), which is present in the *HTTLPR* long (l) allele (rs25531). Call rate was 96.5%. Concordance between DNA replicates showed an accuracy of 100%. All *lg* alleles were recoded into *s*', because it has been shown that this polymorphism represents low serotonin expression comparable to the *s*' allele, while *la* was recoded as *l*' (Nederhof et al., 2010). The 48 bp direct repeat polymorphism in exon 3 of *DRD4* was genotyped on the Illumina BeadStation 500 platform (Illumina.). Three percent blanks as well as duplicates between plates were taken along as quality controls during genotyping. Determination of the length of the alleles was performed by direct analysis on an automated capillary sequencer (ABI3730, Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands) using standard conditions. Call rate for *DRD4* was 99.4%. The 30bp variable number of tandem repeat polymorphism (called *MAOA-LPR* or *MAOA-uVNTR*) was also genotyped on the Illumina BeadStation 500 platform. Three percent blanks as well as duplicates between plates were taken along as quality controls during genotyping. Call rate was 100% for *MAOA*. All polymorphisms were well within Hardy-Weinberg equilibrium (HWE *p* values ranged from 0.77 to 0.87).

Cumulative Genetic Plasticity Index

Each polymorphism was assigned one point if a plasticity genotype was present and these values were summed to create a cumulative index. Girls who were heterozygous for the *MAOA* gene, which is located on the X chromosome, were categorized into the low activity (i.e., high plasticity) genotype as proposed by Belsky et al. (Belsky & Beaver, 2011). Three groups were created according to the number of plasticity genotypes present: a low group (high plasticity in 0–3 genes), intermediate group (high plasticity in 4–5 genes), and a high group (high plasticity in 6–8 genes). Initially, the low group was defined as individuals carrying 0 to 1 plasticity genotypes, but because of insufficient power (n = 22) we merged this group with the individuals carrying two or three plasticity genotypes. The three groups were of unequal size, with the intermediate group having the most individuals. We expected the intermediate group to be the most heterogeneous with regard to each of the individual genes, therefore we deemed necessary to create relatively equally sized groups for the high and lowest plasticity groups.

Covariates

Gender and socioeconomic status (SES) were included as covariates. Five standardized variables (professional occupation and educational attainment for both father and mother, and household income) were averaged and used to calculate SES. The lowest 25% of scores were considered as "low SES," the highest 25% as "high SES," and the remaining were grouped as "intermediate SES."

Analysis

Imputation of missing data. A detailed account of attrition rates within the TRAILS study can be found elsewhere (Huisman et al., 2008). Multiple imputations were performed for all variables except for the missing genotyping data (N = 1,196 at all three measurement waves) using STATA version 10 (STATA corp., College Station, TX). Five different datasets (Rubin, 1996) were created and were subsequently imported into Mplus 5 (Muthén & Muthén, 2007) to be used in the analysis.

Cross-lagged path model. A cross-lagged path model (Structural Equation Modeling) using Mplus was created to investigate cross-sectional and prospective associations between PA and depressive symptoms. Using this model we estimated the direction of the prospective effect (cross-lagged) between PA and depressive symptoms but also the autoregressive paths (relative stability over time) and the cross-sectional covariances (interpreted as correlations at T1 and as correlated change at T2 and T3). Correlated change and cross-lagged paths reflect longitudinal relationships. Because of positive skewness of the depression variables, they were log transformed prior to analysis. The cross-lagged path model was also adjusted for gender and SES. A Comparative Fit Index (CFI) and Tucker-Lewis Index (TLI) greater than 0.95 and a Root Mean Square Error of Approximation (RMSEA) lower than 0.05 were considered a good model fit.

Cumulative plasticity index and individual genes comparisons. A cross-lagged path model was specified for the three groups based on the cumulative plasticity index. To test whether there was a group difference in the reciprocal associations between PA and depressive symptoms, we compared a model where the cross-sectional paths at T1 and the cross-lagged paths were constrained to be equal between groups with a model where these paths were allowed to differ. To test whether the individual polymorphisms affect the reciprocal association between PA and depressive symptoms a similar design was used for each of the individual genes. A χ^2 difference test was performed to test whether the differences between the models reached statistical significance. To avoid possible chance findings because of multiple testing, a significant χ^2 difference score at the .01 level was considered a significant difference between groups.

Results

Descriptive Statistics

Descriptive statistics of the main variables used in this study are shown in Table 1. Both depressive symptoms and PA remained stable over time. Frequencies for the cumulative plasticity index and the frequencies for the different genotypes in individual genes are shown in Table 2.

Relationships Between Depression and PA

Relationships between the total Depressive symptoms score and PA are shown in Figure 1 (overall model fit, $\chi^2 = 6.445$, $p > .05$, $df = 6$). Fit indices showed an excellent fit of model (CFI = 0.999, TLI = 0.999, RMSEA = 0.008), which was significantly better than the fit of the model without cross-lagged paths from PA to depressive symptoms (χ^2 difference = 6.825, $df = 1$. $p < .05$) and

Table 1
*Means (*SD*) of the Main Variables Used in This Study (*N = *1,196)*

	T1 (mean age = 11.1)	T2 (mean age = 13.5)	T3 (mean age = 16.3)
Total depressive symptoms	0.21 (0.19)	0.19 (0.21)	0.21 (.25)
Physical activity	3.52 (2.09)	3.38 (1.68)	3.51 (1.85)

the model without cross-lagged paths from depressive symptoms to PA (χ^2 difference = 8.333, $df = 1$, $p < .05$). As in our previous article with more participants, all cross-lagged paths were significant, indicating that depressive symptoms at any wave predicted PA at the next and vice versa. The autoregressive estimate of depression was higher than the autoregressive estimate of PA. The cross-sectional association (and correlational change) between PA and depression was significant at all assessment waves.

Multigroup Analysis

The multigroup analysis for the three different putative plasticity groups (low, intermediate, and high) is shown in Table 3. Because the middle group was expected to be more heterogeneous with regard to each of the individual genes, we first compared the highest group against the lowest group (χ^2 unconstrained = 9.456, $df = 12$, χ^2 constrained = 16.897, $df = 16$) and then compared all groups with each other (χ^2 unconstrained = 16.709, $df = 18$, χ^2 constrained = 24.728, $df = 26$). The fit indices for both models were excellent (CLI = .998, TLI = .995, RMSEA = .015). As seen in Table 3, the χ^2 difference test was not significant at the .01 level.

Individual Polymorphisms

We carried out the same multigroup analysis approach for each of the individual polymorphisms, comparing the putative plasticity alleles against the nonplasticity alleles. Table 3 depicts the χ^2 difference test for each individual polymorphism. No significant differences were found, just a trend ($p = .03$) for *MAOA*.

Discussion

The results of this study demonstrate reciprocal associations between PA and depressive symptoms consistent with previous studies (Lindwall et al., 2011; Stavrakakis et al., 2011) in larger samples. This suggests that early engagement in PA precedes a decrease in later depressive symptoms and vice versa.

This was the first study using a cumulative plasticity index as proposed by Belsky et al. as a moderator in the association between PA and depressive symptoms. According to Belsky and Pluess, the cumulative plasticity index assumes an additive effect of these genes, where the susceptibility to environmental influences is hypothesized to increase with increasing numbers of plasticity alleles. These genes play a role, through the translation and transcription of specific proteins, in the formation and degradation of serotonin and dopamine. Both of these neurotransmitters are affected by PA and physical inactivity. They also have been implicated in the etiology of depression, therefore making them primary candidates for modifying the reciprocal association between PA and depressive symptoms. However, in the current study, we did not find support for any modifying effects of the

Table 2
Frequencies of the Plasticity Index and Individual Genes

	Groups (*N* = 1,196)		
	Low group (0–3 plasticity alleles)	Intermediate group (4–5 plasticity alleles)	High group (6–8 plasticity alleles)
Plasticity index	28.7%	52.6%	18.7%
Individual genes		Genotype distribution (%)	
BDNF	Val/Val	Val/Met*	Met/Met*
	62.5%	34.4%	3.1%
DRD2	G/G	A/G*	A/A*
	63%	33%	4%
COMT	A/A	A/G*	G/G*
	30.2%	50.1%	19.7%
DRD4	s/s	s/l*	l/l*
	63%	32.3%	4.7%
5-HTR2A	C/C	C/T*	T/T*
	41%	44.6%	14.4%
5-HTTLPR	l/l	l/s*	s/s*
	26.2%	50%	23.8%
TPH1	C/C	C/A*	A/A*
	36.5%	48.7%	14.8%
MAOA girls ($n = 625$)	High	Intermediate*	Low*
	19.4%	55.2%	25.4%
MAOA boys ($n = 571$)	High	Low*	N/A
	37.1%	62.9%	

* Indicates which genotypes were used as the plasticity genotype(s).

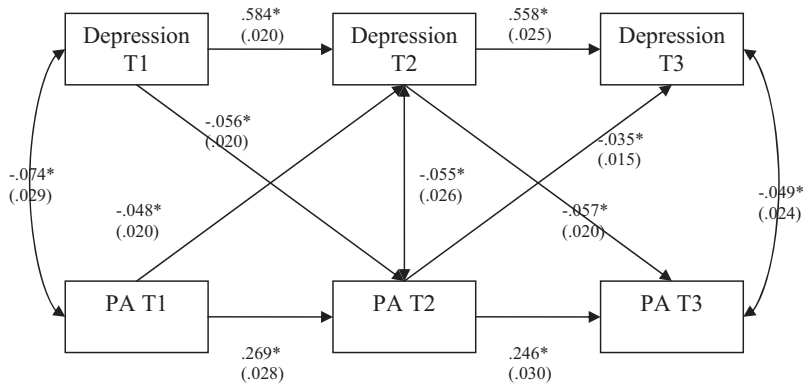

Figure 1. Associations between depressive symptoms and physical activity (PA) over time. The path coefficients reflect model estimated standardized beta values. The effects are adjusted for gender and socioeconomic status (SES). * $p < .05$.

cumulative plasticity index on the associations between PA and depressive symptoms.

We then proceeded in investigating the role of individual polymorphisms on the association of PA and depressive symptoms. To date, there are only three published studies investigating modifying effects of specific individual polymorphisms on the association between PA and depressive symptoms (Mata et al., 2010; Rethorst et al., 2010; Rethorst et al., 2011). The two studies by Rethorst and colleagues investigated the *5-HTTLPR* polymorphism, while the study by Mata and colleagues investigated the *BDNF* polymorphism. All three studies found an influence of specific genotypes (the carriers of met allele for *BDNF* and the carriers of the short allele for *5-HTTLPR*) on the relationship between PA and depressive symptoms. None of these findings were confirmed in our study. Both the *BDNF* and *5-HTTLPR* genotypes as well as the other individual genes that we investigated did not modify the reciprocal association between PA and depressive symptoms in our sample.

This lack of modifying effect could be down to a number of reasons. First, perhaps these plasticity genes are not influencing the relationship between PA and depressive symptoms at all, contrary to our expectations. It is possible that other genes acting on different neurotransmitter systems, such as the endorphin system (Fox, 1999; Hegadoren, O'Donnell, Lanius, Coupland, & Lacaze-Masmonteil, 2009) or the endocannabinoid system (Sparling, Giuffrida, Piomelli, Rosskopf, & Dietrich, 2003), might be playing a more important role in influencing the relationship between PA and depressive symptoms. Second, it is possible that plasticity genes play a role on the association between PA and depressive symptoms but the design chosen in this study was not appropriate to reveal these effects. This could be either because of measurement error (see later section on strengths and limitations for further information) or because the measurement waves were not close enough to capture the processes involved in the interrelationships of plasticity, PA and depressive symptoms. In animal research the ability to remain active (through free wheel running) is thought to be an enriching environment with numerous benefits on behavior and physiology. In humans, PA can be considered a supportive or an enriching experience, especially when individuals start exercising from a very early age and find it a pleasurable experience, but that is still a matter of debate. In our study, we did not find an effect of early engagement in exercise (at the age of 12) on depressive symptoms and vice versa in individuals carrying more plasticity genotypes compared to individuals carrying less. However, to investigate this plasticity theory in depth, the effects of even earlier (early childhood) exercise participation on depres-

Table 3
Multigroup Analysis Comparing the Three Different Cumulative Plasticity Groups (Low, Intermediate, and High) and Individual Polymorphisms

	χ^2diff = χ^2con. − χ^2uncon.	*df* diff = *df* con. − *df* uncon.	*p*
Polymorphism			
Cumulative plasticity (high vs. low of 3 groups)	7.44	4	.11
Cumulative plasticity (3 groups)	8.02	8	.43
Individual polymorphisms			
BDNF	3.03	4	.55
5-HTTLPR	7.12	4	.13
DRD2	2.50	4	.64
MAOA	10.75	4	.03
5-HTR2A	3.64	4	.46
COMT	5.82	4	.21
DRD4	4.23	4	.38
TPH1	8.41	4	.08

sive symptoms should be investigated in individuals carrying these plasticity genotypes. Finally, the exact functioning of certain polymorphisms is not entirely clear. For example, with respect to the *TPH1* gene previous studies have produced mixed results of which allele might be acting as the plasticity allele. Belsky et al. (2009) assumed that carrying the A allele is the putative plasticity genotype while other studies have suggested that the A allele might be playing a protective role against MDD irrespective of positive environmental influences (Viikki et al., 2010). *MAOA* is another example of this complexity. The *MAOA* gene is only found at the X chromosome, which makes the grouping of females more troublesome than the grouping for males.

Further research on the exact function of these genes on the neurotransmitter systems and the effects of exercise on the human biology is needed in order to discover important genetic and biological markers of the association between PA and depressive symptoms. Epistatic interactions (gene-gene) must be also studied extensively. Although studies on epistasis might be complicated and difficult to conduct, the results might be informative in relation to individual differences behind the reciprocal associations between PA and depressive symptoms.

There are some notable strengths in this study. First, the sample size which was considerably larger than previous studies mentioned. Second, the longitudinal design (3 measurements) allowed for the detection and investigation of changes between PA and depressive symptoms over a long period of time (6 years). Finally, the model fit was excellent, modeling the reciprocal relationship between PA and depressive symptoms in our data with great accuracy.

However, there are also limitations. First, most of the analysis relied on self-reports. Self-reports are not as reliable as more objective measurements of PA, such as, calculation of Metabolic Equivalent of Task (METs) in minutes by accelerometers. In addition, PA was assessed by a single question which was slightly different at T1 than at the last two waves, unlike validated PA questionnaires (e.g., IPAQ) which include a series of detailed questions of PA involvement. At T2 and T3 separate questions for winter and summer were asked and more response categories were used compared to T1. Moreover, information on the nature (leisure-time, voluntary or spontaneous), intensity or duration of PA were not collected. This type of information may prove useful for a better understanding of the influence of specific genes on the association between PA and depressive symptoms, since different types, intensities and durations of PA have been shown to be differentially associated with depressive symptoms. Furthermore, because of insufficient power, we merged the group without any plasticity alleles ($n = 22$) with the group carrying one to three alleles, probably leading to some information loss. Finally, it would be optimal to investigate changes in neurotransmitter levels as a result of engaging in PA or increases/decreases in depressive symptoms before investigating genetic modification, in order to advance the knowledge of how these effects (PA and depressive symptoms) affect human biology.

In conclusion, although gene-environment and studies on epistasis can produce conflicting results and seem overly complicated, there is still optimism that these studies can shed light on important genetic factors which might explain individual differences in the associations between PA and depressive symptoms. This elucidation of genetic differences between individuals is necessary for designing effective treatments for affective disorders. However, at this time we did not find support for the notion that plasticity genes may moderate the reciprocal association between depressive symptoms and PA.

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